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Title: Dysfunctional Skin-Derived Glucocorticoid Synthesis Is a Novel Pathogenic Mechanism of Psoriasis

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Short Title: Dysfunctional GC Synthesis in Psoriasis.

ABSTRACT:

Glucocorticoids (GC) are the primary steroids that regulate inflammation and have been exploited therapeutically in inflammatory skin diseases. Despite the broad-spectrum therapeutic use of GC the biochemical rationale for locally treating inflammatory skin conditions is poorly understood, as systemic GC production remains largely functional in these patients. GC synthesis has been well characterised in healthy skin but the pathological consequence has not been examined. Here we show *de novo* GC synthesis and GR expression is dysfunctional in both non-lesional and lesional psoriatic skin. Use of GR epidermal knockout (GREKO) mice with adrenalectomy allowed for the distinction between local (keratinocyte) and systemic GC activity. Compensation exhibited by adult GREKO mice demonstrated that keratinocyte-derived GC synthesis protected skin from topical PMA-induced inflammatory assault. Thus localized *de novo* GC synthesis in skin is essential for controlling inflammation and loss of the GC pathway in psoriatic skin forms a novel pathological process in this complex inflammatory skin disease.

INTRODUCTION

Psoriasis affects 2% of the World's population, of which 80% suffer from mild forms of the disease that are commonly treated with topical therapies (Uva et al. 2012). Glucocorticoids (GC), often in combination with vitamin D or retinoids, are the primary topical therapeutics for mild-moderate psoriasis. GC, released as cortisol (humans) and corticosterone (rodents), are stress-response hormones with potent immunoregulatory mechanisms (Lightman et al. 2008). GC can suppress keratinocyte inflammation, downregulate proliferation and promote differentiation (Stojadinovic et al. 2006). Since GC form an integral component of many topical psoriasis therapies, and healthy keratinocytes can synthesise GC *de novo* (Cirillo and Prime 2011; Hannen et al. 2011; Vukelic et al. 2011; et al. 2016), we hypothesise that loss of effective skin-GC synthesis forms a novel pathogenic mechanism of psoriasis.

Systemically, GC are produced via the hypothalamic-pituitary-adrenal (HPA) axis, forming an integral circadian rhythm and acutely in response to stress. In psoriasis, the systemic HPA axis remains largely unperturbed (Karanikas et al. 2009) and psoriasis patients are not routinely prescribed systemic GC treatment. Healthy skin has a localized HPA axis, expressing all the components for regulating and synthesizing GC (Wierzbicka et al. 2016; Slominski et al. 2015; Jozic et al. 2015; Skobowiat and Slominski 2015; Slominski et al. 2014; Skobowiat et al. 2011; Zmijewski et al. 2007; Slominski et al. 2007; Ito et al. 2005); however the rate of GC synthesis is <1% of classic steroidogenic tissue (Slominski et al. 2004, 2015) thus the significance relative to the abundant systemic supply from adrenal glands remains contested (Jozic et al. 2015).

Psoriasis is primarily considered an immune condition but precisely why the immune system targets skin in psoriasis, and is activated following environmental stress is unclear. The skin-HPA axis has been suggested as a stress-response mechanism (Reich et al. 2010; Slominski et al. 2013) and healthy human keratinocytes respond to stressors such as humidity (Takei et al. 2013), UV light (Skobowiat and Slominski 2015) and trauma (Vukelic et al. 2011) by *de novo* synthesizing GC. Thus defective GC synthesis in psoriasis skin could form an additional mechanism to explain uncontrolled inflammation within lesional tissue and heightened sensitivity to disease flare from environmental cues. Here we assess the necessity of localized GC synthesis in skin independently of the adrenal gland, and its pathological consequences in psoriasis.

RESULTS

Steroid enzyme expression is reduced in psoriatic keratinocytes

RNA relative expression analysis of enzymes required for *de novo* cortisol synthesis were mined from (Nair et al. 2009) using the NCBI GEO profiling database (http://www.ncbi.nlm.nih.gov/geo/) and revealed decreased StAR and 3βHSD1 ($P<0.0001$) in psoriasis skin (figure 1A). We previously reported an absence StAR expression in psoriatic skin (Hannen et al. 2011) but show here that 3βHSD1 protein was absent in non-lesional and lesional psoriatic epidermis. Additionally, CYP11A1 and CYP17 protein was significantly reduced in lesional psoriatic skin, although there was a small increase in CYP11A1 RNA expression in the psoriatic lesion (figure 1B). Gene expression of 11βHSD1 and 11βHSD2, responsible for cortisol to cortisone interconversion, were also significantly reduced in lesional tissue (figure 1A).

De novo cortisol synthesis is suppressed in psoriatic keratinocytes

De novo cortisol synthesis in primary healthy and psoriatic keratinocytes was assessed by [^{3}H]-pregnenolone radiometric assay (figure 1C). More than 40% [^{3}H]-pregnenolone was metabolized to cortisol in healthy primary keratinocytes, confirmed by co-migration with an authentic [1,2,6,7-^{3}H]-cortisol standard. Less than 10% of pregnenolone was metabolized to cortisol in uninvolved and involved psoriatic keratinocytes (figure 1D). There was little metabolism of [^{3}H]-pregnenolone in involved keratinocytes. In uninvolved psoriatic keratinocytes, metabolic intermediates were still detected together with increased formation of highly polar steroid species correlating with sulfated steroids (figure 1C and figure S1). Steroid sulfotransferase SULT2B1 was significantly elevated in uninvolved and involved psoriasis skin relative to healthy controls (figure S1). Sulfate conjugation is an excretion mechanism for steroid clearance (Mueller et al. 2015) and could account for the depletion in cortisol bioavailability in uninvolved psoriasis skin.

Cortisol secretion by whole-skin mounts, from normal and paired uninvolved and involved psoriatic skin, was assessed by LC-MS/MS. Cortisol secretion was decreased by >90% in uninvolved and involved lesional psoriasis skin compared to healthy skin (healthy skin 809.6±120.4 ng/ml; uninvolved 63.1±7.9 ng/ml; involved 67.7±11.8 ng/ml psoriatic skin; $P<0.01$, n=8) (figure 1E). Cortisone levels were also significantly ($P<0.001$) lower in psoriatic tissue (normal skin 1940.0±436.3 ng/ml, uninvolved 272.5±211.5 ng/ml; involved 320.6±101.4 ng/ml psoriatic skin) (figure 1F); indicating that decreased cortisol levels did not occur via shifts in 11βHSD1 shuttle activity. If changes in the direction of 11βHSD1 activity were responsible for depleted cortisol levels, a concomitant increase in cortisone levels would be expected, which was not observed here. The cortisol and cortisone was ratio still 1:2 respectively in both normal and

psoriatic systems. Instead, these data suggest that decreased *de novo* synthesis with steroid sulfation is responsible for dramatic cortisol reduction in psoriatic keratinocytes.

Glucocorticoid receptor levels and activity are impaired in psoriatic keratinocytes

We assessed GR and active (GC/ligand-bound) phospho-Ser^{211} GRα (pGR) to determine whether decreased local cortisol production was associated with decreased GR-phosphorylation. Relative expression analysis identified a significant reduction of GR in lesional psoriatic skin compared to healthy controls and non-lesional skin (figure 2A). However, immunoblotting and immunofluorescence histochemistry (IFH) showed there was a significant (P<0.05) down-regulation of pGR and GR expression in uninvolved psoriatic skin (figure 2). GR was undetectable in 18 of the 20 involved psoriatic skin biopsies by IFH and was significantly (P<0.05) reduced in immunoblotting analysis (figure 2). Notably, there was no difference between uninvolved and healthy skin at mRNA level, thus reduction of GR protein expression in uninvolved tissue is either due to changes of translation or degradation. Since GR expression is essential for epidermal barrier integrity, reduced expression of GR in psoriatic skin could directly affect barrier function.

Skin-derived *de novo* glucocorticoid synthesis prevents topical PMA-mediated inflammation

To examine the specific importance of local keratinocyte cortisol synthesis and associated GR response, we utilized keratinocyte GR knockout mice (GREKO; K5-cre//GR$^{loxP/loxP}$). Multiple keratinocyte steroid enzyme-knockouts would be required to achieve the same effect as knocking out the GR to locate the GC-specific response, thus the GREKO mouse was more suitable for this initial study. To separate local from systemic GR responses, control (CON; 0cre//GR$^{loxP/loxP}$) and GREKO mice were adrenalectomised (ADX) or sham-operated (Sham), to create four groups; (1)

CON-Sham; (2) CON-ADX; (3) GREKO-Sham; (4) GREKO-ADX. Mice were topically administered PMA (8 μg) to one side of the dorsal skin to examine differences in inflammation.

In the absence of PMA, there was little phenotypic difference and no difference in epidermal thickness in adult (week 15) CON and GREKO mouse skin, regardless of ADX (figure 3A). In the presence of PMA, male adult CON-Sham and CON-ADX epidermis was severely disrupted with epidermal thickening and immune cell infiltration (Figure 3A); 50% of CON-Sham and 60% of CON-ADX mice exhibited regions of complete epidermal destruction. In comparison, GREKO-Sham showed no significant epidermal thickening relative to untreated controls and only one GREKO-ADX mouse exhibited increased skin thickness, depicted in the scatter plot distribution (figure 3Aii). Epidermal destruction was not detected in GREKO-Sham and was only observed in 25% GREKO-ADX mice (Figure 3Aii). Inflammatory profiles of male CON PMA-treated dorsal skin showed induction of IL-1β, IL-6, TNFα and MCP1 mRNA levels (figure 3B). In particular, PMA elevated IL-1β 30-fold in CON-sham and 70-fold in CON-ADX male animals, whereas GREKO-Sham and GREKO-ADX mice were protected from PMA-mediated inflammation.

Unlike male CON-sham mice, the inflammatory profile of female CON-sham mice was not elevated upon PMA application, although the skin did appear hyperproliferative (Figure S2). PMA increased epidermal thickness in all groups but no complete epidermal destruction was observed in any of the female groups. However, two out of three female CON-ADX animals died within 12 hours of PMA application and the inflammatory profile of the surviving female mouse measured IL-1β 60-fold higher (Figure S2). In contrast, all the female GREKO-ADX animals survived, did not exhibit excessive IL-1β induction and were protected from immune cell

infiltration. A similar profile was detected with TNFα, which was 8-fold higher in PMA CON-ADX female mice. Therefore, some sexual dichotomy was observed, although female adult GREKO mice still exhibited protection from PMA-induced inflammation since they survived the duration of the experiment.

The protective effects observed in adult GREKO mice was surprising since we hypothesized that mice lacking keratinocyte GR would have an over-active inflammatory response. To understand mechanisms underlying the protective inflammatory phenotype in adult GREKO mice, we assessed systemic and skin corticosterone (CORT) levels. Adult GREKO mice displayed a normal AM-PM circadian rhythm (Figure 4A), a critical process for normal systemic CORT regulation and secretion. CORT levels were markedly reduced in CON-ADX mice, an effect that was maintained 7 days post-ADX, demonstrating the effectiveness of the procedure (Figure 4B). In contrast to CON-ADX, GREKO-ADX mice displayed similar levels of CORT to Sham mice (Figure 4B).

Localized GC synthesis is elevated in adult GREKO mouse skin

LC-MS/MS analysis revealed elevated CORT levels in skin of adult GREKO-sham mice relative to CON-sham mice (figure 4C). Although CORT levels were significantly reduced in CON-ADX mice as expected, there was still measurable amounts of CORT in the skin. Whole skin CORT levels in GREKO-ADX animals were three-fold higher compared to CON-ADX mice and were not significantly different from CON-sham mice. This demonstrates a measurable amount of CORT in skin from a non-adrenal source. Key regulators of the HPA axis (CRH and POMC mRNA and CYP11A1 protein) were significantly elevated in GREKO compared to CON mouse

skin (Figure 4 D-F). Hence, although GR was knocked out in GREKO epidermis, skin synthesis of CORT was not compromised and was elevated to compensate for the loss of GR in keratinocytes. The compensatory local GC supply in adult GREKO mice was sufficient to protect the skin from PMA-induced immune cell infiltration (that express GR) through paracrine signaling, even in the absence of the adrenal glands. Thus local CORT can have a physiological influence on keratinocyte/epidermal function that is distinct from systemic CORT. These data demonstrate that local *de novo* GC synthesis is present and adaptable in skin to protect from inflammation. Since the GC pathway is severely compromised in psoriatic skin, the loss of this pathway could contribute to the exacerbated inflammatory response observed in psoriasis.

DISCUSSION

GC are powerful anti-inflammatory compounds and are the primary therapeutic for the majority of inflammatory skin conditions (Rahman et al. 2012). Although *de novo* GC synthesis has been well researched in healthy skin (Ito et al. 2005; Hannen et al. 2011; Vukelic et al. 2011; Cirillo and Prime 2011; Skobowiat et al. 2011; Nejati et al. 2013; Slominski et al. 2014), there is little appreciation for the pathophysiological necessity of localized GC production in skin, especially since the systemic HPA axis can supply GC in abundance to most tissues. Psoriasis patients are rarely treated with systemic GC and their HPA axis remains largely intact (Harbuz et al. 2003; Buske-Kirschbaum et al. 2006), although a subpopulation of psoriasis patients showed mild HPA axis perturbations during acute social stress (Richards et al. 2005) and psychological stress has been associated with skin barrier breakdown and cutaneous infections (Aberg et al. 2007; Slominski 2007). The common use of topical GC-based therapies indicates there is a local depletion of GC in psoriasis skin.

Here we show that localized GC production is virtually ablated in psoriasis skin. Reduced bioavailability of cortisol was observed in uninvolved and lesional psoriasis skin, due to decreased synthesis and increased steroid sulfation, demonstrating that effective stress response is lost even in uninvolved skin. In the absence of a stressor, loss of skin GC production did not independently induce an inflammatory response, but localized GC production in mouse skin was found to prevent PMA-induced inflammation independently of the adrenal gland. Thus, defective *de novo* GC synthesis, from steroid enzymes to receptor, provides a novel pathogenic mechanism of psoriasis.

StAR controls acute steroid synthesis and is downregulated in psoriasis, aging skin and other inflammatory skin disorders (Tiala et al. 2007; Hannen et al. 2011; Manna et al. 2015; Slominski et al. 2015). Whilst StAR is the rate-determining protein for steroidogenesis, simple observations of alterations in StAR expression do not provide information on the type of steroid being produced or non-StAR mediated steroidogenesis. Steroid production is also regulated by MLN64, which is expressed abundantly in differentiating keratinocytes (Watari et al. 1997; Bose et al. 2000; Hannen et al. 2011). Thus the absence of StAR does not necessarily equate to a complete loss of steroid production in skin, however, our study shows GC production is clearly defective in psoriasis in addition to depleted StAR expression.

GR expression was also ablated in lesional psoriatic keratinocytes. In contrast, another study detected GR in lesional psoriasis skin but found it failed to translocate into the cell nucleus (Man et al. 2013). However, this study used a Han Chinese cohort thus patients were of a different ethnicity to the UK-based study presented here. There are four GR SNP haplotypes that can

regulate GC sensitivity that are also associated with psoriasis (Stevens et al. 2003). Investigations are required to determine whether psoriasis patients can be stratified according to their GR expression levels or localised steroidogenic capacity.

The GREKO mouse model exhibited compensation in the form of elevated skin GC. Age-dependent GC effects are not uncommon, since GC can improve barrier formation in fetal animals but adversely affect barrier permeability in adult animals (Aszterbaum et al. 1993; Kao et al. 2003). Neonate GREKO mouse exhibited a strong inflammatory, hyperproliferative epidermal phenotype akin to inflammatory skin disease (Sevilla et al. 2012). The skin architecture normalized at 4 weeks in postnatal GREKO mice. By P56 adult GREKO mice had no obvious phenotype but were sensitive to topical PMA treatment (Sevilla et al. 2012). Adult GREKO mice (P105) in this study retained a normal skin phenotype together with protection from topical PMA.

The GREKO-ADX mouse model determined physiological significance of local versus systemic GC response. Upregulated GC synthesis in adult (P105) GREKO mouse skin was consistent with loss of the GC negative feedback response caused by the absence of GR expression (Reich et al. 2010; Slominski et al. 2013a). Whilst GREKO mice keratinocytes are unable to respond to GC via the GR, neighboring skin cells and infiltrating immune cells that ubiquitously express GR will still detect keratinocyte paracrine GC signals. Elevated localized GC in adult GREKO skin was sufficient to protect from PMA-induced inflammation and immune cell infiltration, preventing an inflammatory cytokine storm leading to epidermal destruction.

Elevated skin-HPA axis in adult GREKO mouse also had additive effects to systemic amounts of GC, since plasma levels of GC remained high in GREKO mice even after ADX. Similar findings were observed in *hypophysectomised* mice, which had increased skin and plasma CORT levels, that was further upregulated in the skin upon UV light stimulation (Skobowiat et al. 2011; Skobowiat and Slominski 2015). Mouse epidermis is relatively a larger organ than human epidermis therefore GC production in mouse skin may form a greater contribution to systemic GC levels than in humans. Nevertheless, the evidence here demonstrates skin can influence systemic GC levels, supporting a complex interplay between local and systemic GC regulation (Jozic et al. 2015).

Our findings highlight impaired keratinocyte *de novo* cortisol synthesis as a crucial factor in psoriasis pathogenesis and a novel therapeutic target for disease treatment. Future research will identify mechanisms that rescue endogenous keratinocyte GC synthesis in psoriatic skin; consider whether the pathway could be used to stratify patients prone to GC rebound; and to determine whether GC *de novo* synthesis is disrupted in other skin conditions commonly treat with GC.

MATERIALS AND METHODS

Supplementary information online contains an extended description of materials and methods.

Skin Specimens and Cell Culture- Normal human skin from routine plastic surgery and punch biopsies (4 mm) from uninvolved and involved skin of psoriasis patients were obtained with informed written consent. The East London and City Health Authority Research Ethics Committee

approved the use of skin samples (09/HO704/69). Primary human keratinocytes were isolated and cultured as previously described (Hannen et al. 2011)

Radiometric conversion assays- Normal, psoriatic uninvolved, psoriatic involved primary human keratinocytes ($2x10^5$ cells/ml in 24 well plates) and assay blanks (no cells) were washed three times and incubated with 90.0 pmol [7-^{3}H]-pregnenolone in 1 ml/well EpiLife without supplements, 24h. Steroid isolation and thin layer chromatography were conducted as described previously (Hannen et al. 2011).

LC-MS/MS - Skin specimens ($2mm^2$) were cultured for 24 h in 200 µl EpiLife (no supplements). Culture media was centrifuged and 180 µl of supernatant was spiked with internal standards (IS): 1.37 nmol/L d4-cortisol (Sigma Aldrich, Poole, U.K.) and 0.96 nmol/L d2-cortisone (C/D/N Isotopes Inc., Quebec, Canada). Chloroform (1ml) was added to each sample and mixed vigorously. Alternatively, 30 mg of ventral mouse skin was homogenized with 1ml chloroform containing 1.14 nmol/L d8-corticosterone (C/D/N Isotopes Inc., Quebec, Canada). All samples were centrifuged, the chloroform layer was collected and evaporated under nitrogen gas at 45°C. Dried extracts were reconstituted with 100 µL methanol and subsequently vortexed with 150 µL of water. An aliquot (45 µL) was injected using a CTC-PAL auto-sampler (Presearch) coupled to a Prominence XR HPLC (Shimadzu) and API5500 mass spectrometer (ABSciex).

Chromatographic separation was completed on a pentafluorophenyl-propyl (PFP) column (Ascentis Express F5 2.7 µm 100x2.1 mm; Supelco) using different chromatographic gradients and mass spectrometer sources for the analysis of cortisol and cortisone, and corticosterone.

Cortisol/cortisone mobile phases were A: 20 mM ammonium formate pH3; B: methanol + 0.1% formic acid. Formate adducts of each analyte were detected using electrospray ionisation in negative ion mode. Corticosterone mobile phases consisted of A: 0.1% formic acid in water; B: 0.1% formic acid in methanol. Corticosterone was detected using atmospheric pressure chemical ionisation in positive ion mode.

Immunoblotting sample preparation and analysis- Epidermis was separated from dermis after incubation with 2.5 mg/ml dispase II, 45 min, 37°C. Epidermal protein was isolated in lysis buffer (7 M urea, 2 M thiourea, 4% (v/v) CHAPS, 30 mM Tris-HCl, pH 8.5) for 10 min. Protein samples were prepared for SDS-PAGE/western blotting as outlined previously (Hannen et al. 2011).

Immunofluorescence histochemistry- Standard IFH techniques were used to stain formaldehyde fixed, paraffin embedded sections (0.5 μm). Primary antibody: rabbit anti-pGR (Cell Signaling Technology, Danvers, MA) 4°C overnight, followed by mouse anti-GR (Autogen Bioclear, Calne, U.K.), 1h RT. Alexa Fluor 488 goat anti-mouse and goat anti-rabbit IgG (2 μg/ml; 1h; RT). Leica DM 5000B fluorescence microscope with a Leica DFC 350FX camera and Leica QWin Pro software were used for imaging.

Mouse Bilateral Adrenalectomy-CON (15) and GREKO (22) mice (Sevilla et al. 2012) were maintained on a 12 hr dark/light cycle with *ad libitum* access to food (standard diet) and water. Procedures were conducted on adult mice (15 weeks) at times corresponding to the circadian nadir of the animals' diurnal rhythm and in accordance with European legislation (2007/526/CE). Mice underwent either bilateral adrenalectomy under general anesthetic (ADX), or identical operations

with the adrenals left intact (Sham) (Kim Green, 1974). Mice received subcutaneous analgesic (Rimadyl-120µg/ml), glucose/saline (NaCl 0.45% (w/v), glucose 2.5% (w/v)) to aid recovery. ADX animals also received 0.9% (w/v) saline drinking water. Dorsal fur was shaved 4 days post-surgery and 10 µg PMA (Sigma) was applied topically to the right-side dorsal skin 24h later, the left side was an untreated control. Animals were sacrificed 7 days post-operation; plasma and dorsal skin was collected, ventral skin hair was removed with depilatory cream (Veet), washed thoroughly and snap frozen for LC-MS/MS analysis.

Radioimmunoassay-was conducted following manufacturer's instructions (Cort RIA kit, Izoto, Hungary) to determine CORT levels in mouse plasma (Waite et al. 2012).

Haemotoxylin and Eosin- of mouse skin sections were performed (Sevilla et al. 2012); epidermal thickness was quantified by measuring multiple epidermal regions (Image J line tool).

qRT-PCR- Dorsal mouse skin RNA was extracted using a *RNeasy Lipid* Tissue Mini Kit (Qiagen). Reverse transcription was performed using a Superscript III first strand cDNA synthesis kit (Invitrogen). PCR (40 cycles) was performed with SYBR Green PCR MasterMix (ABgene) or by Taqman-based methodology on an Applied Biosystem 5700 detection system. Relative mRNA levels were determined by standard curve methodology normalized against 18S ribosomal control RNA (Applied Biosystems, Warrington, UK). Changes in gene expression are represented as fold change relative to 1, where control (CON-sham) equals 1. See supplemental information for primer details.

Statistical Analysis- Statistical evaluations were performed using GraphPad Prism 6 (La Jolla, CA) and analyzed by one-way analysis of variance or student *t* test; error bars are mean ± SEM.

CONFLICT OF INTEREST

MP received funding from Giuliani Sp.A. to conduct this research.

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FIGURE LEGENDS

Figure 1. *De novo* corticosteroid synthesis pathway is downregulated in uninvolved and involved psoriatic epidermis. (**A**). STARD1, CYP11A1, CYP17A1, 3βHSD1, CYP21A2 and CYP11B1 relative expression analysis mined from (Nair et al. 2009). (**B**) CYP11A1, 3βHSD1 and CYP17 western analysis from healthy epidermis, uninvolved and involved psoriatic epidermis (n=4). (**C**) Representative thin layer chromatography chromatograms of [^{3}H]-pregnenolone metabolism by healthy controls, uninvolved and involved psoriatic primary keratinocytes over 24h. (**D**) Conversion of [^{3}H]-pregnenolone to cortisol calculated from TLC chromatograms (n=4, P<0.01). (**E**) Cortisol and (**F**) cortisone release from healthy and psoriatic *ex vivo* skin mounts measured by LC-MS/MS (n=8, P<0.001). Uninvolved psoriasis (PSORU U), involved psoriasis (PSOR I); P5 pregnenolone, F cortisol, S-S sulfated steroid. Data expressed as mean ± SEM, * P <0.05, ** P <0.01, ***P<0.001.

Figure 2. Downregulation of GR expression in uninvolved and involved psoriatic skin. (**A**) Relative expression analysis of NR3C1 in healthy epidermis, uninvolved and involved psoriatic epidermis. (**B**) Representative images of immunohistochemical analysis of GR and pGR expression in the epidermis of normal, uninvolved psoriatic and involved psoriatic epidermis (n= 20). (**C**) Quantification of (**i**) GR and (**ii**) pGR expression levels in healthy, uninvolved and involved psoriatic epidermis by densitometry of western immunoblots (n = 4, P<0.05). Uninvolved psoriasis (PSOR U), involved/lesional psoriasis (PSOR I). Data are expressed as mean ± SEM. Statistically significant differences between groups are indicated by: * P <0.05, ** P <0.01, ***P<0.001.

Figure 3. GREKO male mice are protected from the PMA inflammatory response. (**Ai**) H&E analysis of male skin from control (CON) and GREKO mice treated with and without PMA. Mice were either sham operated or adrenalectomised (ADX). (Aii) Quantification of epidermal thickness in CON and GREKO mice and percentage of mice exhibiting complete epidermal destruction following topical PMA treatment. (**B**) qPCR inflammatory cytokine profile (TNFα, IL-6, IL-1, MCP1) of skin from sham and ADX male CON and GREKO mice that were treated topically with or without PMA. Data are expressed as mean ± SEM. Statistically significant differences between groups are indicated by: * P <0.05, ** P <0.01, ***P<0.001. CON male sham n=4, CON male ADX n=5, GREKO male sham n=4, GREKO male ADX n=4.

Figure 4. Corticosterone analysis revealed an upregulation of the skin HPA axis in male GREKO mice. (**A**) AM and PM plasma CORT measured by RIA demonstrate a normal circadian rhythm in GREKO mice. (**B**) Post-adrenalectomy plasma CORT was significantly reduced in CON mice but was not significantly reduced in GREKO ADX mice. (**C**) LC-MS/MS analysis of CORT was significantly higher in GREKO ADX mouse ventral skin than CON ADX ventral mouse skin, indicating a localised skin supply of CORT. Relative expression of (**D**) CRH, (**E**) POMC , (**F**) CYP11A1 in GREKO mouse skin by qPCR; (**I**) elevated CYP11A1 protein expression in male GREKO mice. Data are expressed as mean ± SEM. Statistically significant differences between groups are indicated by: * P <0.05, ** P <0.01. CON male sham n=4, CON male ADX n=5, GREKO male sham n=4, GREKO male ADX n=4.